

WE CLAIM:

1. An oligonucleotide containing:

(a) a first nucleotide sequence,

(b) a second nucleotide sequence at the 5' end of the first nucleotide sequence,

5 (c) a third nucleotide sequence at the 5' end of the second nucleotide sequence, and

(d) a molecular energy transfer trio including an energy donor moiety, and first and second energy acceptor moieties,

wherein:

10 the energy donor moiety is capable of emitting a quantum of energy, and the first and second acceptor moieties are each capable of absorbing a substantial amount of the quantum of energy,

the donor moiety is attached to a nucleotide of the first nucleotide sequence, the first acceptor moiety is attached to a nucleotide of the first nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the third nucleotide sequence; or the  
15 donor moiety is attached to a nucleotide of the third nucleotide sequence, the first acceptor moiety is attached to a nucleotide of the third nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the first nucleotide sequence,

the oligonucleotide is capable of forming a hairpin containing a nucleotide of the first nucleotide sequence and a nucleotide of the third nucleotide sequence, and

20 if the donor moiety emits the quantum of energy, then:

(1) the first acceptor moiety absorbs a substantial amount of the emitted quantum of energy if the hairpin is not formed, and

(2) the second acceptor moiety absorbs a substantial amount of the emitted quantum of energy if the hairpin is formed.

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2. The oligonucleotide of claim 1, wherein the first acceptor moiety absorbs the substantial amount of the emitted quantum of energy only if the hairpin is not formed.

30 3. The oligonucleotide of claim 1, wherein the second acceptor moiety absorbs the substantial amount of the emitted quantum of energy only if the hairpin is formed.

4. The oligonucleotide of claim 1, comprising a deoxyribonucleotide.

5. The oligonucleotide of claim 1, further comprising a fourth nucleotide sequence at the 3' end of the first nucleotide sequence.

6. The oligonucleotide of claim 5, wherein the third nucleotide sequence is not  
5 complementary to the fourth nucleotide sequence.

7. The oligonucleotide of claim 5, wherein the fourth nucleotide sequence is complementary to a nucleotide sequence flanking a target nucleotide sequence.

10 8. The oligonucleotide of claim 7, wherein the target nucleotide sequence is DNA.

9. The oligonucleotide of claim 1, wherein the donor moiety is a fluorophore.

10. The oligonucleotide of claim 1, wherein the first acceptor moiety is a  
15 fluorophore.

11. The oligonucleotide of claim 9, wherein the second acceptor moiety is a quencher of light emitted by the fluorophore.

12. The oligonucleotide of claim 1, wherein the first acceptor moiety is capable of  
20 emitting another quantum of energy.

13. The oligonucleotide of claim 1, wherein the donor moiety is fluorescein.

14. The oligonucleotide of claim 1, wherein the first acceptor moiety is ROX.  
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15. The oligonucleotide of claim 1, wherein the second acceptor moiety is  
DABSYL.

16. The oligonucleotide of claim 1, wherein there is (are) 0 to 50 nucleotide(s) in  
30 between the nucleotide to which the donor moiety is attached and the nucleotide to which the first acceptor moiety is attached.

17. The oligonucleotide of claim 16, wherein there are 5 to 10 nucleotides in between the nucleotide to which the donor moiety is attached and the nucleotide to which the first acceptor moiety is attached.

5        18. The oligonucleotide of claim 1, wherein there is (are) 0 to 50 nucleotide(s) in between the nucleotide to which the donor moiety is attached and the nucleotide to which the second acceptor moiety is attached.

10        19. The oligonucleotide of claim 18, wherein there are 5 to 10 nucleotides in between the nucleotide to which the donor moiety is attached and the nucleotide to which the second acceptor moiety is attached.

15        20. The oligonucleotide of claim 1, wherein, if the hairpin is formed, then the nucleotide to which the donor moiety is attached is the complement of the nucleotide to which the second acceptor moiety is attached.

20        21. The oligonucleotide of claim 1, wherein, if the hairpin is formed, then there is (are) 0 to 5 nucleotide(s) in between the nucleotide to which the donor moiety is attached and the complement of the nucleotide to which the second acceptor moiety is attached.

25        22. An oligonucleotide comprising the nucleotide sequence of SEQ ID NO:1, wherein fluorescein is attached to the nucleotide at position 1 of SEQ ID NO:1, ROX is attached to the nucleotide at position 21 of SEQ ID NO:1, and DABSYL is attached to the nucleotide at position 5 or 10 of SEQ ID NO:1.

23. The oligonucleotide of claim 22 consisting of the nucleotide sequence of SEQ ID NO:1.

30        24. A kit comprising the oligonucleotide of claim 1 and a polymerase.

25. The kit of claim 24, wherein the polymerase is a DNA polymerase.

26. A method for determining if a target nucleotide sequence is present in a sample comprising:

(a) contacting the sample with an oligonucleotide containing:

(1) a first nucleotide sequence,

5 (2) a second nucleotide sequence at the 5' end of the first nucleotide sequence,

(3) a third nucleotide sequence at the 5' end of the second nucleotide sequence, and

10 (4) a molecular energy transfer trio including an energy donor moiety, and first and second energy acceptor moieties,

wherein:

the donor moiety is capable of emitting a first quantum of energy,

the first and second acceptor moieties are each capable of absorbing a substantial amount of the first quantum of energy,

15 the first acceptor moiety is capable of emitting a second quantum of energy,

the donor moiety is attached to a nucleotide of the first nucleotide sequence, the first acceptor moiety is attached to a nucleotide of the first nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the third nucleotide sequence; or the first donor moiety is attached to a nucleotide of the third nucleotide sequence, the first acceptor moiety is attached to a nucleotide of the third nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the first nucleotide sequence,

20 the oligonucleotide is capable of forming a hairpin containing a nucleotide of the first nucleotide sequence and a nucleotide of the third nucleotide sequence, and

25 if the donor moiety emits the first quantum of energy, then the first acceptor moiety absorbs a substantial amount of the emitted first quantum of energy if the hairpin is not formed, and the second acceptor moiety absorbs a substantial amount of the emitted first quantum of energy if the hairpin is formed, and

30 (b) if the second quantum of energy is detected, then determining that the target nucleotide sequence is present in the sample; or if the second quantum of energy is not detected, then determining that the target nucleotide sequence is not present in the sample.

27. The method of claim 26, wherein the first acceptor moiety absorbs the substantial amount of the emitted first quantum of energy only if the hairpin is not formed.

28. The method of claim 26, wherein the second acceptor moiety absorbs the substantial amount of the emitted first quantum of energy only if the hairpin is formed.

29. The method of claim 26, wherein the donor moiety is fluorescein.

30. The method of claim 26, wherein the first acceptor moiety is ROX.

31. The method of claim 26, wherein the second acceptor moiety is DABSYL.

32. A method for determining if a target nucleotide sequence is present in a sample comprising:

(a) contacting the sample with an oligonucleotide containing:

(1) a first nucleotide sequence,

(2) a second nucleotide sequence at the 5' end of the first nucleotide sequence,

(3) a third nucleotide sequence at the 5' end of the second nucleotide sequence, and

(4) a molecular energy transfer trio including an energy donor moiety, and first and second energy acceptor moieties,

wherein:

the donor moiety is capable of emitting a first quantum of energy,

the first and second acceptor moieties are each capable of absorbing a substantial amount of the first quantum of energy,

the first acceptor moiety is capable of emitting a second quantum of energy,

the donor moiety is attached to a nucleotide of the first nucleotide sequence, the first acceptor moiety is attached to a nucleotide of the first nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the third nucleotide sequence; or the first donor moiety is attached to a nucleotide of the third nucleotide sequence, the first acceptor moiety is attached to a

nucleotide of the third nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the first nucleotide sequence,

the oligonucleotide is capable of forming a hairpin containing a nucleotide of the first nucleotide sequence and a nucleotide of the third nucleotide sequence, and

5 if the donor moiety emits the first quantum of energy, then the first acceptor moiety absorbs a substantial amount of the emitted first quantum of energy if the hairpin is not formed, and the second acceptor moiety absorbs a substantial amount of the emitted first quantum of energy if the hairpin is formed,

(b) incorporating the oligonucleotide into a double-stranded nucleic acid if the target  
10 nucleotide sequence is present in the sample, thereby preventing the hairpin from forming,

(c) optionally conducting an amplification reaction, thereby incorporating the oligonucleotide into an amplification product if the target nucleotide sequence is present in the sample, and

(d) if the second quantum of energy is detected, then determining that the target  
15 nucleotide sequence is present in the sample; or if the second quantum of energy is not detected, then determining that the target nucleotide sequence is not present in the sample.

33. The method of claim 32, wherein the first acceptor moiety absorbs the substantial amount of the emitted first quantum of energy only if the hairpin is not formed.

20 34. The method of claim 32, wherein the second acceptor moiety absorbs the substantial amount of the emitted first quantum of energy only if the hairpin is formed.

35. The method of claim 32, wherein the oligonucleotide further comprises a  
25 fourth nucleotide sequence at the 3' end of the first nucleotide sequence.

36. The method of claim 35, wherein the third nucleotide sequence is not complementary to the fourth nucleotide sequence.

37. The method of claim 35, wherein the fourth nucleotide sequence is  
30 complementary to a nucleotide sequence flanking the target nucleotide sequence.

38. The method of claim 32, wherein the donor moiety is fluorescein.

39. The method of claim 32, wherein the first acceptor moiety is ROX.

40. The method of claim 32, wherein the second acceptor moiety is DABSYL.

5        41. The method of claim 32, wherein, in (b), the oligonucleotide is incorporated into the double-stranded nucleic acid using a polymerase.

42. A method for detecting a target nucleotide sequence comprising:

10        (a) annealing a first oligonucleotide to a nucleotide sequence flanking a target nucleotide sequence, wherein the first oligonucleotide contains:

          (1) a first nucleotide sequence,

          (2) a second nucleotide sequence at the 5' end of the first nucleotide sequence,

15        (3) a third nucleotide sequence at the 5' end of the second nucleotide sequence, and

          (4) a molecular energy transfer trio including an energy donor moiety, and first and second energy acceptor moieties,

          wherein:

          the donor moiety is capable of emitting a first quantum of energy,

20        the first and second acceptor moieties are each capable of absorbing a substantial amount of the first quantum of energy,

          the donor moiety is attached to a nucleotide of the first nucleotide sequence, the first acceptor moiety is attached to a nucleotide of the first nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the third nucleotide sequence; or the donor moiety is attached to a nucleotide of the third nucleotide sequence, the first acceptor moiety is attached to a nucleotide of the third nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the first nucleotide sequence,

25        the first oligonucleotide is capable of forming a hairpin containing a nucleotide of the first nucleotide sequence and a nucleotide of the third nucleotide sequence, and

30        if the donor moiety emits the first quantum of energy, then the first acceptor moiety absorbs a substantial amount of the emitted first quantum of

energy if the hairpin is not formed, and the second acceptor moiety absorbs a substantial amount of the emitted first quantum of energy if the hairpin is formed,

(b) extending the 3' end of the first oligonucleotide using the target nucleotide sequence as a template to form an extended first strand, wherein the target nucleotide sequence is annealed to the extended first strand,

(c) separating the target nucleotide sequence from the extended first strand,

(d) annealing a second oligonucleotide to the extended first strand,

(e) extending the 3' end of the second oligonucleotide using the extended first strand as a template to form an extended second strand, wherein the extended first strand is annealed to the extended second strand,

(f) optionally amplifying the extended first and second strands, and

(g) detecting a second quantum of energy emitted by the first acceptor moiety to detect the target nucleotide sequence.

43. The method of claim 42, wherein the first acceptor moiety absorbs the substantial amount of the emitted first quantum of energy only if the hairpin is not formed.

44. The method of claim 42, wherein the second acceptor moiety absorbs the substantial amount of the emitted first quantum of energy only if the hairpin is formed.

45. The method of claim 42, wherein (f) comprises:

(1) separating the extended first strand from the extended second strand,

(2) annealing the first oligonucleotide to the extended second strand, and annealing the second oligonucleotide to the extended first strand,

(3) extending the 3' end of the first oligonucleotide using the extended second strand as a template to form another extended first strand, wherein the extended second strand is annealed to the other extended first strand; and extending the 3' end of the second oligonucleotide using the extended first strand as a template to form another extended second strand, wherein the extended first strand is annealed to the other extended second strand, and

(4) repeating (1), (2), and (3) for a finite number of times, wherein, in (1), the extended first and second strands respectively are the extended first strand and the other



extended second strand of (3), or respectively are the other extended first strand and the extended second strand of (3).

46. The method of claim 42, wherein the oligonucleotide further comprises a  
5 fourth nucleotide sequence at the 3' end of the first nucleotide sequence.

47. The method of claim 46, wherein the third nucleotide sequence is not complementary to the fourth nucleotide sequence.

10 48. The method of claim 46, wherein the fourth nucleotide sequence is complementary to the nucleotide sequence flanking the target nucleotide sequence.

49. The method of claim 42, wherein the donor moiety is fluorescein.

15 50. The method of claim 42, wherein the first acceptor moiety is ROX.

51. The method of claim 42, wherein the second acceptor moiety is DABSYL.

52. A method for detecting a target nucleotide sequence comprising:

20 (a) annealing a first oligonucleotide to a nucleotide sequence flanking a target nucleotide sequence, wherein the first oligonucleotide contains:

(1) a first nucleotide sequence complementary to the nucleotide sequence flanking the target nucleotide sequence, and

25 (2) a second nucleotide sequence at the 5' end of the first nucleotide sequence,

(b) extending the 3' end of the first oligonucleotide using the target nucleotide sequence as a template to form an extended first strand, wherein the target nucleotide sequence is annealed to the extended first strand,

(c) separating the target nucleotide sequence from the extended first strand,

30 (d) annealing a second oligonucleotide to the extended first strand,

(e) extending the 3' end of the second oligonucleotide using the extended first strand as a template to form an extended second strand, wherein the extended first strand is annealed to the extended second strand,

(f) separating the extended first strand from the extended second strand,  
(g) annealing a third oligonucleotide to the extended second strand, wherein the third oligonucleotide contains:

(1) a first nucleotide sequence,

5 (2) a second nucleotide sequence at the 5' end of the first nucleotide sequence,

(3) a third nucleotide sequence at the 5' end of the second nucleotide sequence,

10 (4) a fourth nucleotide sequence at the 3' end of the first nucleotide sequence, and

(5) a molecular energy transfer trio comprising an energy donor moiety, and first and second energy acceptor moieties,

wherein:

15 the fourth nucleotide sequence is complementary to the complement of the second sequence of the first oligonucleotide,

the donor moiety is capable of emitting a quantum of energy,

the first and second acceptor moieties are each capable of absorbing a substantial amount of the quantum of energy,

20 the donor moiety is attached to a nucleotide of the first nucleotide sequence, the first acceptor moiety is attached to a nucleotide of the first nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the third nucleotide sequence; or the donor moiety is attached to a nucleotide of the third nucleotide sequence, the first acceptor moiety is attached to a nucleotide of the third nucleotide sequence, and the second acceptor moiety is attached to a  
25 nucleotide of the first nucleotide sequence,

the third oligonucleotide is capable of forming a hairpin containing a nucleotide of the first nucleotide sequence and a nucleotide of the third nucleotide sequence, and

30 if the donor moiety emits the quantum of energy, then the first acceptor moiety absorbs a substantial amount of the emitted quantum of energy if the hairpin is not formed; and the second acceptor moiety absorbs a substantial amount of the emitted quantum of energy if the hairpin is formed,

(h) extending the 3' end of the third oligonucleotide using the extended second strand as a template to form a doubly extended first strand, wherein the doubly extended first strand is annealed to the extended second strand,

5 (i) separating the doubly extended first strand from the extended labeled second strand,

(j) annealing the second oligonucleotide to the doubly extended first strand,

(k) extending the 3' end of the second oligonucleotide using the doubly extended first strand as a template to form a doubly extended second strand, wherein the doubly extended first strand is annealed to the doubly extended second strand,

10 (l) optionally amplifying the doubly extended first and second strands, and

(m) detecting a second quantum of energy emitted by the first acceptor moiety to detect the target nucleotide sequence.

15 53. The method of claim 52, wherein the first acceptor moiety absorbs a substantial amount of the emitted quantum of energy only if the hairpin is not formed.

54. The method of claim 52, wherein the second acceptor moiety absorbs a substantial amount of the emitted quantum of energy only if the hairpin is formed.

20 55. The method of claim 52, wherein (l) comprises:

(1) separating the doubly extended first strand from the doubly extended second strand,

(2) annealing the second oligonucleotide to the doubly extended first strand, and annealing the third oligonucleotide to the doubly extended second strand,

25 (3) extending the 3' end of the second oligonucleotide using the doubly extended first strand as a template to form another doubly extended second strand, wherein the doubly extended first strand is annealed to the other doubly extended second strand; and extending the 3' end of the third oligonucleotide using the doubly extended second strand as a template to form another doubly extended first strand, wherein the doubly extended  
30 second strand is annealed to the other doubly extended first strand, and

(4) repeating (1), (2), and (3) for a finite number of times, wherein, in (1), the doubly extended first and second strands respectively are the doubly extended first strand

and the other doubly extended second strand of (3), or respectively are the other doubly extended first strand and the doubly extended second strand of (3).

56. The method of claim 52, wherein the third nucleotide sequence is not  
5 complementary to the fourth nucleotide sequence.

57. The method of claim 52, wherein the fourth nucleotide sequence is  
complementary to the nucleotide sequence flanking the target nucleotide sequence.

10 58. The method of claim 52, wherein the donor moiety is fluorescein.

59. The method of claim 52, wherein the first acceptor moiety is ROX.

60. The method of claim 52, wherein the second acceptor moiety is DABSYL.

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61. A method for determining if a first or second target nucleotide sequence is  
present in a sample comprising:

(a) contacting the sample with:

(1) a first oligonucleotide containing:

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(i) a first nucleotide sequence,

(ii) a second nucleotide sequence at the 5' end of the first  
nucleotide sequence,

(iii) a third nucleotide sequence at the 5' end of the second  
nucleotide sequence, and

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(iv) a molecular energy transfer trio including a first energy  
donor moiety, and first and second energy acceptor moieties,

wherein:

the first donor moiety is capable of emitting a first quantum of  
energy,

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the first and second acceptor moieties are each capable of  
absorbing a substantial amount of the first quantum of energy,

the first donor moiety is attached to a nucleotide of the first  
nucleotide sequence, the first acceptor moiety is attached to a nucleotide

of the first nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the third nucleotide sequence; or the first donor moiety is attached to a nucleotide of the third nucleotide sequence, the first acceptor moiety is attached to a nucleotide of the third nucleotide sequence, and the second acceptor moiety is attached to a nucleotide of the first nucleotide sequence,

the first oligonucleotide is capable of forming a first hairpin containing a nucleotide of the first nucleotide sequence and a nucleotide of the third nucleotide sequence, and

if the first donor moiety emits the first quantum of energy, then the first acceptor moiety absorbs a substantial amount of the emitted first quantum of energy if the first hairpin is not formed, and the second acceptor moiety absorbs a substantial amount of the emitted first quantum of energy if the first hairpin is formed, and

(2) a second oligonucleotide containing:

(i) a fourth nucleotide sequence,

(ii) a fifth nucleotide sequence at the 5' end of the fourth nucleotide sequence,

(iii) a sixth nucleotide sequence at the 5' end of the fifth nucleotide sequence, and

(iv) a molecular energy transfer pair including a second energy donor moiety and a third energy acceptor moiety,

wherein:

the second donor moiety is capable of emitting a second quantum of energy,

the third acceptor moiety is capable of absorbing a substantial amount of the second quantum of energy,

the second donor moiety is attached to a nucleotide of the fourth nucleotide sequence and the third acceptor moiety is attached to a nucleotide of the sixth nucleotide sequence, or the second donor moiety is attached to a nucleotide of the sixth nucleotide sequence and the third acceptor moiety is attached to a nucleotide of the fourth nucleotide sequence,

the second oligonucleotide is capable of forming a second hairpin containing a nucleotide of the fourth nucleotide sequence and a nucleotide of the sixth nucleotide sequence, and

if the second donor moiety emits the second quantum of energy, then the third acceptor moiety absorbs a substantial amount of the emitted second quantum of energy if the second hairpin is formed,

(b) incorporating:

(1) the first oligonucleotide into a first double-stranded nucleic acid if the first target nucleotide sequence is present in the sample, thereby preventing the first hairpin from forming, and

(2) the second oligonucleotide into a second double-stranded nucleic acid if the second target nucleotide sequence is present in the sample, thereby preventing the second hairpin from forming,

(c) optionally conducting:

(1) a first amplification reaction, thereby incorporating the first oligonucleotide into a first amplification product if the first target nucleotide sequence is present in the sample, and

(2) a second amplification reaction, thereby incorporating the second oligonucleotide into a second amplification product if the second target nucleotide sequence is present in the sample, and

(d) determining that:

(1) the first target nucleotide sequence is present in the sample if a third quantum of energy emitted by the first acceptor moiety is detected, or the first target nucleotide sequence is not present in the sample if the third quantum of energy is not detected, and

(2) the second target nucleotide sequence is present in the sample if a fourth quantum of energy emitted by the third acceptor moiety is detected, or the second target nucleotide sequence is not present in the sample if the fourth quantum of energy is not detected.

62. The method of claim 61, wherein the first acceptor moiety absorbs a substantial amount of the emitted first quantum of energy only if the first hairpin is not formed.

63. The method of claim 61, wherein the second acceptor moiety absorbs a substantial amount of the emitted first quantum of energy only if the first hairpin is formed.

5           64. The method of claim 61, wherein the third acceptor moiety absorbs a substantial amount of the emitted second quantum of energy only if the second hairpin is formed.

10           65. The method of claim 61, wherein the first oligonucleotide further comprises a seventh nucleotide sequence at the 3' end of the first nucleotide sequence.

66. The method of claim 65, wherein the third nucleotide sequence is not complementary to the seventh nucleotide sequence.

15           67. The method of claim 65, wherein the seventh nucleotide sequence is complementary to a nucleotide sequence flanking the first target nucleotide sequence.

20           68. The method of claim 61, wherein the second oligonucleotide further comprises an eighth nucleotide sequence at the 3' end of the fourth nucleotide sequence.

69. The method of claim 67, wherein the sixth nucleotide sequence is not complementary to the eighth nucleotide sequence.

25           70. The method of claim 67, wherein the eighth nucleotide sequence is complementary to a nucleotide sequence flanking the second target nucleotide sequence.

71. The method of claim 61, wherein the first and second donor moieties are each fluorescein.

30           72. The method of claim 61, wherein the first acceptor moiety is ROX.

73. The method of claim 61, wherein the second and third acceptor moieties are each DABSYL.

74. The method of claim 61, wherein, in (b), the first oligonucleotide is incorporated into the first double-stranded nucleic acid using a polymerase, and the second oligonucleotide is incorporated into the second double-stranded nucleic acid using a polymerase.